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Prognostic Evaluation of Cutaneous Malignant Melanoma: A Clinicopathologic and Immunohistochemical Study

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Background and Objectives: Depth of invasion and stage of the disease are established prognostic indicators in cutaneous malignant melanoma. The role of other parameters is still an open problem.

Methods: In 93 consecutive patients with cutaneous malignant melanoma, the level of invasion, tumor thickness, ulceration, vascular invasion, lymphoplasmocytic infiltrates, and mitotic index were evaluated by histology. Expression of Ki-67 and PCNA proliferative antigens together with vimentin, S100, and HMB 45 proteins were assessed by immunohistochemistry.

Results and Conclusions: Disease-free and overall survival were correlated with tumor stage, tumor thickness, level of invasion, macroscopic pattern, ulceration, vascular invasion, expression of HMB 45, PCNA, and Ki-67/MIB1. Stage, HMB 45, and PCNA were independent prognostic factors for disease-free survival, whereas tumor stage, tumor thickness, and expression of both proliferative antigens influenced overall survival independently. The variables studied demonstrated reciprocal correlation; therefore, analysis of many prognostic parameters in malignant melanoma could be recommended. *J. Surg. Oncol. 1999;70:150–160.*

KEY WORDS: **malignant melanoma; stage of the disease; histology; immunohistochemistry; proliferative activity**

INTRODUCTION

Prognostic value of commonly used clinical and morphological parameters in cutaneous malignant melanoma is still an open problem and only tumor thickness and stage of the disease seem to be independent indicators of the clinical outcome [1–13]. Some results point to markers of proliferative activity [14–21] and expression of p53 protein [14,17,22–26] as variables of prognostic value. In the present study, prognostic value of common clinical and histological parameters in cutaneous malignant melanoma together with proliferative activity and some tumor markers have been evaluated.

MATERIAL AND METHODS

Patients

The study was performed on 93 unselected, consecutive patients with primary cutaneous malignant melanoma of the skin, treated in the Center of Oncology in Cracow between January 1983 and August 1991. The patients were sent by general practitioners or from regional hospitals. In none of them was atypical mole phe-

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TABLE I. Antibodies Used in Current Prognostic Evaluation of Malignant Melanoma

Antigen	Clone	Dilution	Source[a]/catalog number
Ki-67	MIB-1	1:1	Immunotech/0607
PCNA	PC10	1:200	DAKO/M0879
p53	DO-7	1:200	DAKO/M7001
S 100		1:1	DAKO/N4573
HMB 45		1:80	Biogenex/MU001-UC
Vimentin	V9	1:200	DAKO/M0725

[a]Immunotech, Marseille, France; DAKO, Glostrup, Denmark; Biogenex, San Ramon, CA.

notype or familial occurrence of malignant melanoma observed. All patients were treated by radical tumor excision; without local lymphadenectomy in 45 cases and in 48 cases with it. Adjuvant chemotherapy was administered after surgery in 46 patients with more advanced tumors. Complete clinical information was obtained in 91 patients; 2 of the more advanced patients treated by tumor excision and local lymphadectomy were lost to follow-up.

Histological Evaluation

Assessment of histology was performed on routine, hematoxylin/eosin-stained 5-μm microtome paraffin sections. The tumors were classified according to the World Health Organization classification [27] and the level of invasion was measured according to Clarke and Breslow as recommended by Levene et al. [28]. The measurements were performed at 50× magnification of the microscope equipped with an eyepiece with a micron scale. The assessment of invasion was carried out only in those tumor parts where the whole epidermal layer was preserved. The same device was also used to estimate the skin margins. After the depth of invasion and nodal status were evaluated by histology, the tumor stage was defined according to the guidelines of the International Union Against Cancer [29].

Lymphoplasmocytic infiltrates of various intensity, vascular invasion, and presence of ulceration, found only microscopically, were also examined. Mitotic figures were counted per 100 tumor cells in at least 10 randomly selected fields at 400× magnification of a Zeiss-Axioscope microscope equipped with an eyepiece with a grid. Then mitotic index was defined as an average number of such countings.

Immunohistochemistry

For immunohistochemical studies, 5-μm sections of tumors were examined by the avidin-biotin-peroxidase complex (ABC) technique using appropriate positive and negative controls throughout. The antibodies used, their source, and their dilution are shown in Table I. The immunohistochemical studies were not performed in all cases because of scarcity of embedded tumor tissue.

TABLE II. Cutaneous Malignant Melanomas: Independent Prognostic Indicators for Disease-Free Survival—Mulitvariate Cox Model Results

Variable	Value	Number of cases	Relative risk	*P* value	5-year survival (%)
Stage	Ia	33	1.00		88.2
	Ib	28	5.05	0.0045	44.0
	II	14	8.11	0.0000	19.0
	III	18	38.54	0.0000	5.6
HMB 45	0%–90%	46	1.00		56.2
	91%–100%	47	1.86	0.0459	41.3
PCNA index	0.00–0.15	20	1.00		84.4
	0.16–0.35	56	4.00	0.0381	47.9
	0.36–1.00	17	4.44	0.0356	8.6

TABLE III. Independent Prognostic Indicators for Overall Survival: Multivariate Cox Model Results

Variable[a]	Value	Number of cases	Relative risk	*P* value	5-year survival (%)
Stage	Ia + Ib	61	1.00		84.3
	II	14	3.23	0.0319	50.0
	III	18	11.86	0.0000	13.9
BLI[a]		93	1.17	0.0104	
PCNA index	0.00–0.35	76	1.00		77.4
	0.36–1.00	17	2.27	0.0338	9.5
Ki-67 index	0.00–0.20	81	1.00		73.0
	0.21–1.00	12	5.17	0.0002	9.3

[a]BLI, depth of invasion according to Breslow considered as a continuous variable.

Positive reactions to S100, HMB 45, and vimentin of various intensity in cytoplasm and cell membrane occurred in different percentages of tumor cells and were considered in three categories: ⩽50%; >50% and ⩽75%; and >75% tumor cells.

Cell nuclei with positive reactions of various intensity for PCNA and Ki-67 antigens were considered as indexes, estimated in the same way as mitotic index. Nuclear reaction to p53 was categorized in five groups as quotient value of staining intensity and the number of positive cells [30].

Statistical Analysis

The differences between mean values have been tested by analysis of variance. The influence of factors on survival has been assessed by log-rank test and Cox proportional hazard model. Multiple models were also estimated by a stepwise selection procedure. Multivariate models presented in Tables II and III have been obtained by stepwise (backwards) selection procedure. Initially, all variables identified as significant through univariate analysis were put into the model. Then nonsignificant variables were eliminated (one at a time) with respect to the highest *P* value. The procedure stops if all variables

TABLE IV. Cutaneous Malignant Melanoma: Location of Tumors

Area	Region	Number of cases
Trunk	Dorsal	22
	Abdominal	4
	Thoracic	5
Lower extremity/hip	Buttock	1
	Thigh	8
	Shank	20
	Sole	5
Upper extremity	Shoulder	2
	Arm	8
	Forearm	4
	Palm	1
Head and neck	Cheek	8
	Scalp	2
	Ear	1
	Neck	2

have *P* value at a satisfactory (low) level. So if a variable is not included in the final multivariate model, it can be considered as nonsignificant.

RESULTS

Age and Sex

The tumors occurred in 58 females and 35 males. The mean age of patients at the diagnosis was 48 years (range, 17–78); 46.9 years (range, 19–78) for females and 50 years (range, 17–76) for males.

Location

Most lesions occurred on the trunk or lower extremity (Table IV) and tumor location was strongly associated with patients gender ($P = 0.0000$); in women it occurred more often on extremities (women, 30/58, 52%, and men, 5/35, 14%), and in men it occurred more often on the trunk (men, 24/35, 69%, and women, 9/58, 16%).

Gross Findings

The tumors studied presented one of four macroscopic patterns: pattern 1, in 14/93 (15.0%) cases irregular, flat, brown, or red-brown macules of mean diameter 16 mm (range, 6–39 mm) were found; pattern 2, 10/93 lesions (10.8%) presented as flat plaques, gray-pinkish or black in color, of mean diameter 11.2 mm (range, 7–24 mm); pattern 3, in 63/93 (67.7%) cases elevated, well-circumscribed nodules, brown or blackish, with or without necrotic foci on the cut surface, with a mean diameter of 18 mm (range, 5–45 mm) were observed; and pattern 4, 6/93 (6.5%) tumors presented as irregular, craterous ulceration with roller-shaped rims and the mean diameter of these lesions was 11.2 mm (range, 8–29 mm). Satellite lesions were observed only in single cases of the 1 and 2 macroscopic melanoma pattern.

TABLE V. Prognostic Evaluation of Cutaneous Malignant Melanoma Patients (n = 93): Tumor Stage

Stage	Number of cases (%)	Mean Breslow thickness in mm (range)	Clarke level (Number of cases)				
			CLI I	CLI II	CLI III	CLI IV	CLI V
Ia	33 (35.5)	1.1 (0.1–1.5)	7	8	18		
Ib	28 (30.1)	4.5 (2.1–8.5)		2	7	19	
II	14 (15.1)	6.9 (2.5–12.0)		1	2	7	4
III	18 (19.4)	7.25 (2.1–13.5)		1	1	7	9

Microscopic Findings

Superficially spreading and lentigo maligna melanoma were observed in 5 and 10 patients, respectively. The most frequent type of the lesion was nodular melanoma that occurred in 78 patients; in 6 cases of nodular melanoma constituents of superficially spreading melanoma were also present. Lentigo maligna accompanied superficially spreading melanoma in two cases and nodular melanoma in one case.

Constituent of benign melanotic lesions was observed in nodular melanoma (lentigo simplex in one case, marginal nevus in two, and blue nevus in one case) and in lentigo maligna melanoma (dermal in two and marginal nevus in one case).

Histological type of melanoma was significantly associated with Breslow depth of invasion ($P = 0.0004$) and Clark level ($P = 0.0000$). In superficially spreading type of melanoma, mean value of Breslow thickness amounted to 0.8 mm; in lentigo maligna melanoma, 1.13 mm; and in nodular melanoma, 4.83 mm. In all tumors of superficially spreading melanoma, Clark level was lower than IV, in 8 cases of 10 lentigo maligna tumors it was lower than III and 33 of 78 cases of nodular melanoma were classified as Clark level IV.

Among patients studied, early tumor stages were more frequent (Table V). In stage Ia macroscopic patterns 1 and 2 were significantly more frequent than in other stages ($P = 0.0008$) and were found in 10 and 6 cases, respectively; pattern 3 was observed in 17 cases. Stage Ia occurred in 5 cases of superficially spreading type, in 9 cases of lentigo maligna, and in 19 of nodular melanoma. The mean width of skin margin amounted to 4.8 mm (range, 2–20 mm).

In stage Ib, macroscopic pattern 1, 2, 3, and 4 was found in 3, 2, 22, and 1 patients, respectively. Lentigo maligna melanoma occurred in 1 case and nodular melanoma in 27 cases. The mean width of skin margin amounted to 6.3 mm (range, 1.0–13.5 mm). Stage II malignant melanomas presented macroscopic pattern 3 in 12

TABLE VI. Prognostic Evaluation of Malignant Melanomas: Histological Lesions

Stage	Ulceration (number of cases)	Vascular invasion (number of cases)	Lymphoplasmocytic infiltration (number of cases)
Ia	14/33 (42.4%)	8/33 (24.2%)	22/33 (66.7%)
Ib	22/28 (78.6%)	18/28 (64.3%)	21/28 (75.0%)
II	12/14 (85.7%)	10/14 (71.4%)	10/14 (71.4%)
III	15/18 (83.3%)	15/18 (83.3%)	13/18 (72.2%)
Total	63	51	66

cases and pattern 4 in 2 cases. The only histological tumor type observed in this stage was nodular melanoma. The mean width of skin margin amounted to 7.1 mm (range, 3–22 mm). Stage III malignant melanomas presented macroscopic patterns 1, 2, 3, and 4 in 1, 2, 12, and 3 cases, respectively. Nodular melanoma was also the only histological type observed in this tumor stage. The mean width of skin margin amounted to 6.7 mm (range, 1.0–17.5 mm).

Tumor stage was interrelated with age ($P = 0.0450$) and demonstrated a tendency to be correlated with patients gender ($P = 0.0844$). In stage III, the mean age of patients was the lowest and amounted to 43 years; 11 of 35 tumors (31%) in men and 7 of 58 tumors (12%) in women were in this stage at the time of diagnosis.

Histological lesions that accompanied malignant melanomas are presented in Table VI. Presence of ulceration and vascular invasion, reciprocally interrelated ($P = 0.0002$), were correlated with tumor stage ($P = 0.0028$ and 0.0002, respectively) and occurred more frequently in higher stages. Ulceration and vascular invasion were positively correlated with increasing Clark level ($P = 0.0005$ and 0.0000, respectively) and Breslow thickness ($P = 0.0003$ and 0.0000, respectively). Vascular invasion was also associated with type of tumor ($P = 0.0023$) and was found in 2/5 of superficially spreading melanoma, 63% (49/78) of nodular melanoma, but in no case of lentigo maligna melanoma (LMM). Presence of ulceration demonstrated only a trend to be interrelated with tumor type ($P = 0.0641$) and occurred in 73% (57/78) of nodular melanoma and in 2/5 and 4/10 of superficially spreading and lentigo maligna melanoma, respectively. Lymphoplasmocytic infiltrates showed no interrelationship with tumor stage ($P = 0.7797$), but were positively correlated with increasing Clark level ($P = 0.0470$), with vascular invasion ($P = 0.0019$) and with type of tumor ($P = 0.0155$). Lymphoplasmocytic infiltrates were found in 2/5 of superficially spreading melanoma, 6/10 of lentigo maligna melanoma, and 74% (58/78) of nodular melanoma.

Immunohistochemistry

Results of S100, HMB 45, and vimentin reactions for all melanomas studied, irrespectively of tumor stage, are presented in Table VII. All of them occurred in most tumor cells. Expression of these markers demonstrated no reciprocal interrelationships. Vimentin in all categories of reaction intensity was inversely correlated with Clark level, stage ($P = 0.0503$ and 0.0005, respectively), and demonstrated a tendency to be associated with vascular invasion ($P = 0.0840$).

TABLE VII. Positive Reactions to S100, HMB 45, and Vimentin in Malignant Melanomas

Antigen	Category (number of cases) 1	2	3
S100	5/92 (5.4%)	7/92 (7.6%)	80/92 (87.0%)
HMB 45	19/93 (20.4%)	21/93 (22.6%)	53/93 (57.0%)
Vimentin	11/77 (14.3%)	12/77 (15.6%)	54/77 (70.1%)

TABLE VIII. Indicators of Proliferative Activity in Malignant Melanomas

Stage	Mean value of indexes (range) PCNA index	Ki-67 index	Mitotic index
Ia	0.18 (0.03–0.40)	0.07 (0.01–0.20)	0.03 (0.00–0.10)
Ib	0.25 (0.02–0.48)	0.11 (0.02–0.35)	0.09 (0.01–0.16)
II	0.31 (0.10–0.61)	0.14 (0.01–0.56)	0.13 (0.01–0.20)
III	0.43 (0.17–0.76)	0.18 (0.02–0.83)	0.06 (0.01–0.15)

Twenty-one cases of 33 tumors in stage Ia, 22 cases of 28 in stage Ib, 11 of 14 in stage II, and 16 of 18 tumors in stage III were p53-positive. In all these cases reaction was intensive (category IV or V). Expression of p53 was not associated with any variable studied.

Proliferative Activity

Mean values of the mitotic, PCNA, and Ki-67 indexes amounted respectively to 0.07 (range, 0.00–0.20), 0.29 (range, 0.02–0.76), and 0.13 (range, 0.01–0.89) for the whole melanoma group studied. The last two indexes demonstrated no correlation. The same data but evaluated for particular tumor stages are presented in Table VIII; only index of PCNA and mitotic index were correlated with tumor stage ($P = 0.0004$ and 0.0047, respectively). Index PCNA was also interrelated with tumor type ($P = 0.0262$); mean values of PCNA index amounted to 0.14 in superficially spreading melanoma, 0.18 in lentigo maligna type, and 0.26 in nodular melanoma. All three indexes demonstrated positive linear correlation with Breslow thickness (mitotic index, $r = 0.209$, $P = 0.0039$; PCNA index, $r = 0.465$, $P = 0.0000$; Ki-67 index, $r = 0.281$, $P = 0.0005$). Index of PCNA was also correlated with Clark level ($r = 0.403$,

TABLE IX. Tumor Relapses in Patients With Cutaneous Malignant Melanoma

Stage	Number of cases	Recurrence[a]						
		L	L + LLN	L + LLN + M	L + M	LLN	LLN + M	M
Ia	6	1				3		2
Ib	17	1	2	1		5	5	3
II	12	3	1		1	3	1	3
III	15		1	4	3		3	4
Total	50	5	4	5	4	11	9	12

[a]L, local; LLN, locoregional lymph nodes; M, metastases.

$P = 0.0000$) and index of Ki-67 demonstrated negative linear correlation with vimentin expression ($r = -0.218$, $P = 0.0310$).

Follow-Up

The mean observation time amounted to 44.7 months (range, 2–116 months). Tumor relapses occurred in 50 patients; detailed description is presented in Table IX. Forty-five patients died (41 of malignant melanoma and 4 of unrelated causes) and of the remaining 46 patients 26 live without symptoms of the disease and 20 with them.

Clinicopathological Correlations

Parameters studied in relation to disease-free and overall survival are summarized in Tables X and XI. For the expression of HMB 45, S100, vimentin, and two proliferative antigens, optimal cutoff points in relation to disease-free and overall survival have been investigated and these variables were considered both as categorized and continuous variables. Expression of HMB 45 is characterized by only one such point (90% of positive cells irrespective of reaction category), index of PCNA by two optimal cutoff points in relation to disease-free survival (0.15 and 0.35, respectively), and one cutoff point for overall survival (0.35). Optimal cutoff point for overall survival for index of Ki-67 amounted to 0.20.

Multivariate analysis revealed that tumor stage, HMB 45 expression, and PCNA index, both considered as categorized variables, influenced disease-free survival independently (Table II). Tumor stage, Breslow thickness (considered as continuos variable), PCNA, and Ki-67 indexes (both assessed as categorized variables) were independent prognostic indicators for overall survival (Table III). Correlations of tumor stage, expression of HMB 45, PCNA index, Ki-67 index, and Breslow thickness with disease-free and overall survival are presented in Figures 1–6.

TABLE X. Influence of Qualitative and Quantitative Categorized Variables on Disease-Free and Overall Survival

Variables	Disease-free survival *P* value (log-rank test)	Overall survival *P* value (log-rank test)
Stage	0.0000	0.0000
Localization	0.9060	0.6275
Sex	0.5253	0.3095
Macroscopic pattern	0.0094	0.0072
Clark level	0.0005	0.0001
Ulceration	0.0062	0.0349
Vascular invasion	0.0010	0.0071
Lymphoplasmocytic infiltration	0.8981	0.8274
HMB 45	0.0259	0.0193
S100	0.2673	0.2533
Vimentin	0.0541	0.0020
p53	0.1454	0.1959
PCNA index	0.0008	0.0002
Ki-67 index	0.0040	0.0003

TABLE XI. Influence of Quantitative Continuous Variables on Disease-Free and Overall Survival

Variables	Disease-free survival *P* value (univariate Cox model)	Overall survival *P* value (univariate Cox model)
Age	0.2182	0.0975
Mininal margin	0.0672	0.0312
Breslow thickness	0.0000	0.0000
Mitotic index	0.0908	0.5405
HMB 45	0.0816	0.1499
S100	0.1967	0.1848
Vimentin	0.0856	0.0082
PCNA index	0.0000	0.0000
Ki-67 index	0.0030	0.0007

DISCUSSION

Clinical Parameters

Prognostic value of age and gender in melanoma patients is a subject of controversy. Some studies revealed less favorable clinical outcome in patients older than 65 years, whose tumors demonstrated morphological indicators of poor prognosis more frequently [3,4,31], other studies did not confirm prognostic importance of age [9,11,13,32]. Females demonstrated better clinical outcome than males, whose survival was shorter [3,4,32], and metastases appeared more frequently at the time of diagnosis [2,6]. Other studies, however, provided different results [11,13]. Anatomic location of lesion can also be of prognostic information; a higher risk of death was

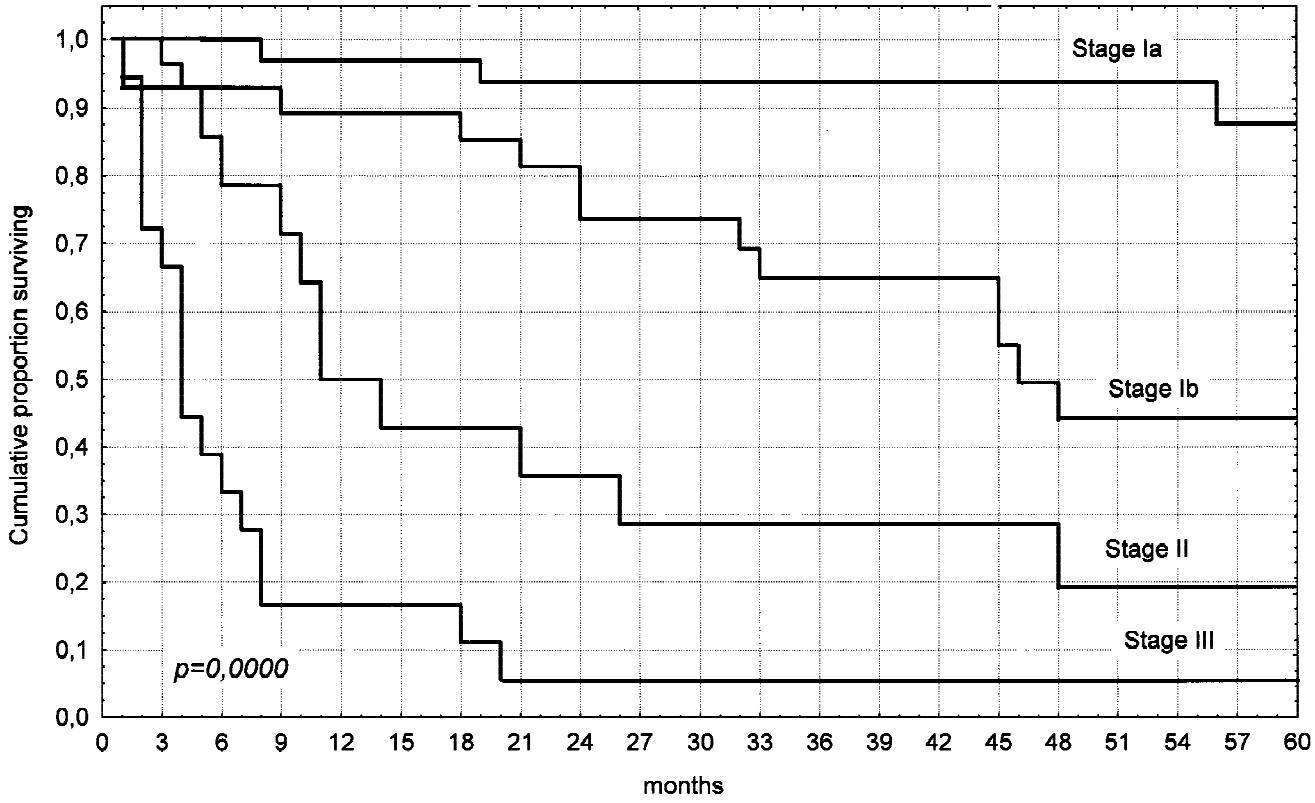

Fig. 1. Disease-free survival curves for 33 patients with stage Ia melanoma, 28 patients with stage Ib, 14 patients with stage II, and 18 patients with stage III. *P* value taken from the log-rank test comparing four survival curves.

associated with tumors located on the back, thorax, upper arm, neck, and scalp [2,4,33–36]. Tumors of the head and neck area, even when thin, recurred rapidly and with higher frequency [37,38]. The anatomic location of lesion was strongly associated with patients' gender; unfavorable trunk location of melanoma was more frequent in males, as demonstrated other studies [5,7].

In our study, cutaneous melanoma occurred more often in females, their age was lower than males, and most lesions were located on the trunk or lower extremity. Age, sex, and tumor location were not associated with disease-free or overall survival. Nevertheless, at the time of diagnosis, higher tumor stages were more frequent in males and patients in high tumor stages constituted the youngest group. This result is concordant with some results, which indicate younger melanoma patients as a group of higher risk [2,6]. Our study pointed also to the macroscopic pattern as a prognostic parameter in malignant melanoma, which in univariate analysis influenced disease-free and overall survival.

Histological and Immunohistochemical Parameters

Invasiveness of cutaneous melanoma expressed as a tumor thickness or a level of invasion is the powerful prognostic indicator in malignant melanoma [1–4,8,34,39]. The present study has confirmed the prognostic importance of Breslow thickness and Clark level; however, only Breslow thickness was an independent predictor for overall survival. This result suggests superiority of Breslow thickness as a clinical predictor, which is in concordance with other studies [8,39]. Prognostic importance of both indicators of tumor invasion is also confirmed by their interrelationship with other variables of predictive value.

The other indicator strongly influencing clinical outcome is a status of locoregional lymph nodes, and the number but not the size of metastatic lymph nodes is of prognostic importance [2,40]. In the study presented, invasiveness of cutaneous melanoma, assessed in Breslow and Clark categories, was considered jointly with nodal status as a tumor stage. This parameter appeared to be an independent predictor of disease-free and overall survival, as confirmed other studies [11,12,41].

Several results point to histological type of malignant melanoma as a prognostic factor [3,13]. In the study presented, nodular melanoma outnumbered other melanoma types and tumor type did not correlate with clinical outcome. Other results also preclude histological type of melanoma as a predictor of clinical outcome [2,10], although large radial or vertical growth component is of prognostic value [12].

Benign melanocytic nevi were observed only in few

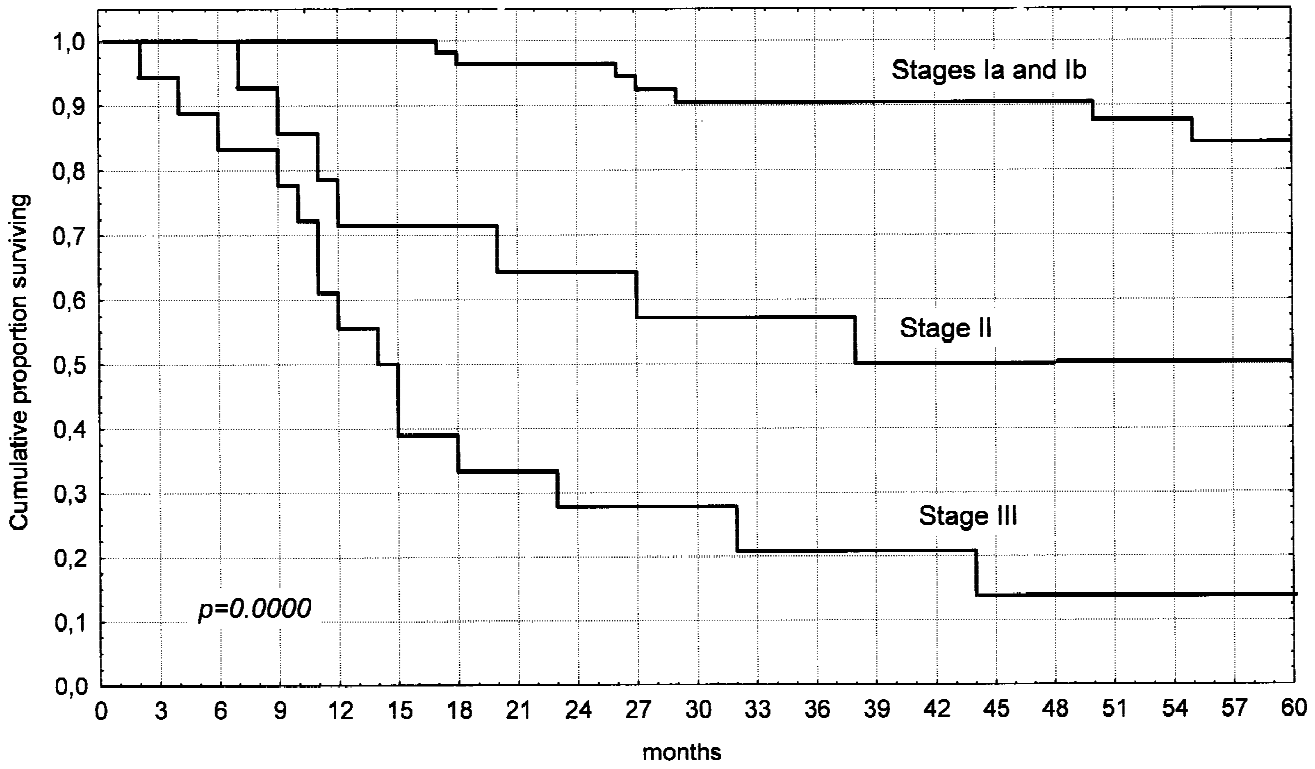

Fig. 2. Overall survival curves for 61 patients in stage Ia and Ib, 14 patients in stage II, and 18 patients in stage III. *P* value taken from log-rank test comparing three survival curves.

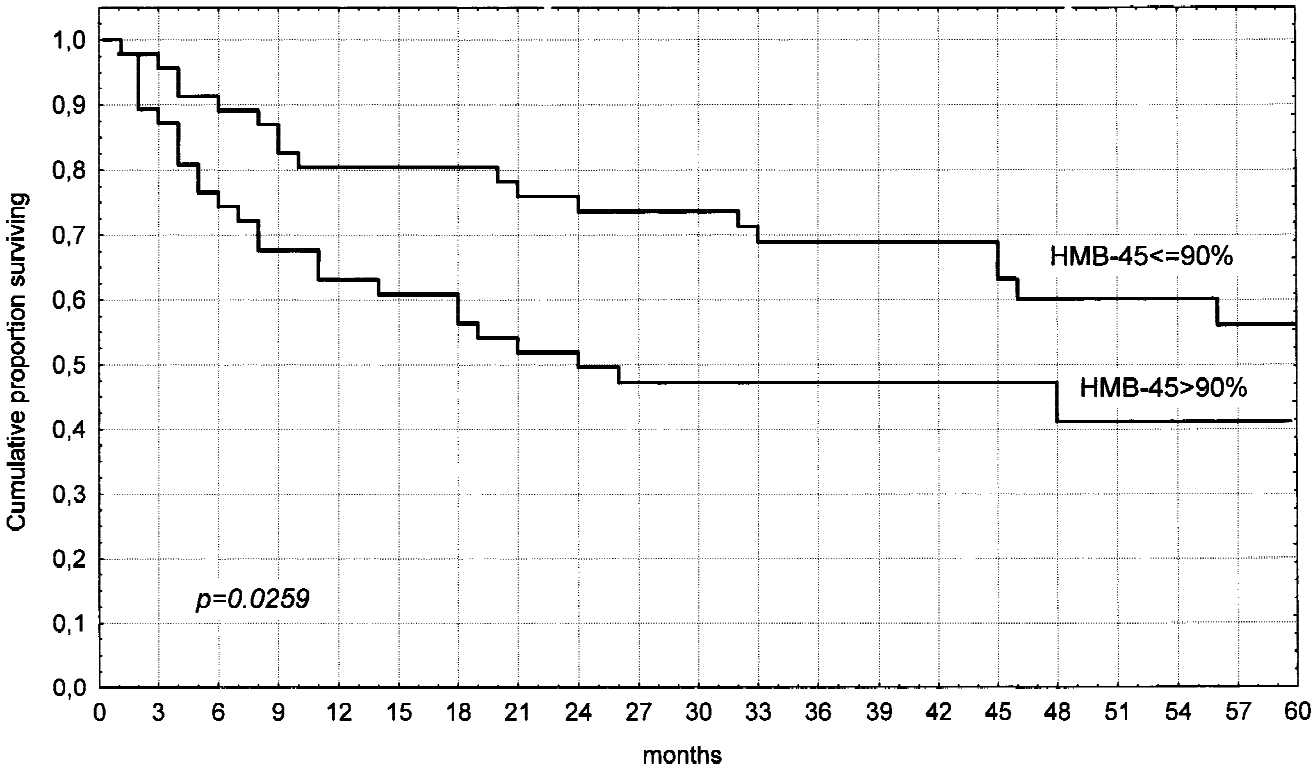

Fig. 3. Disease-free survival curves for 46 patients with HMB ⩽90% and 47 patients with HMB >90%. *P* value taken from log-rank test comparing two survival curves.

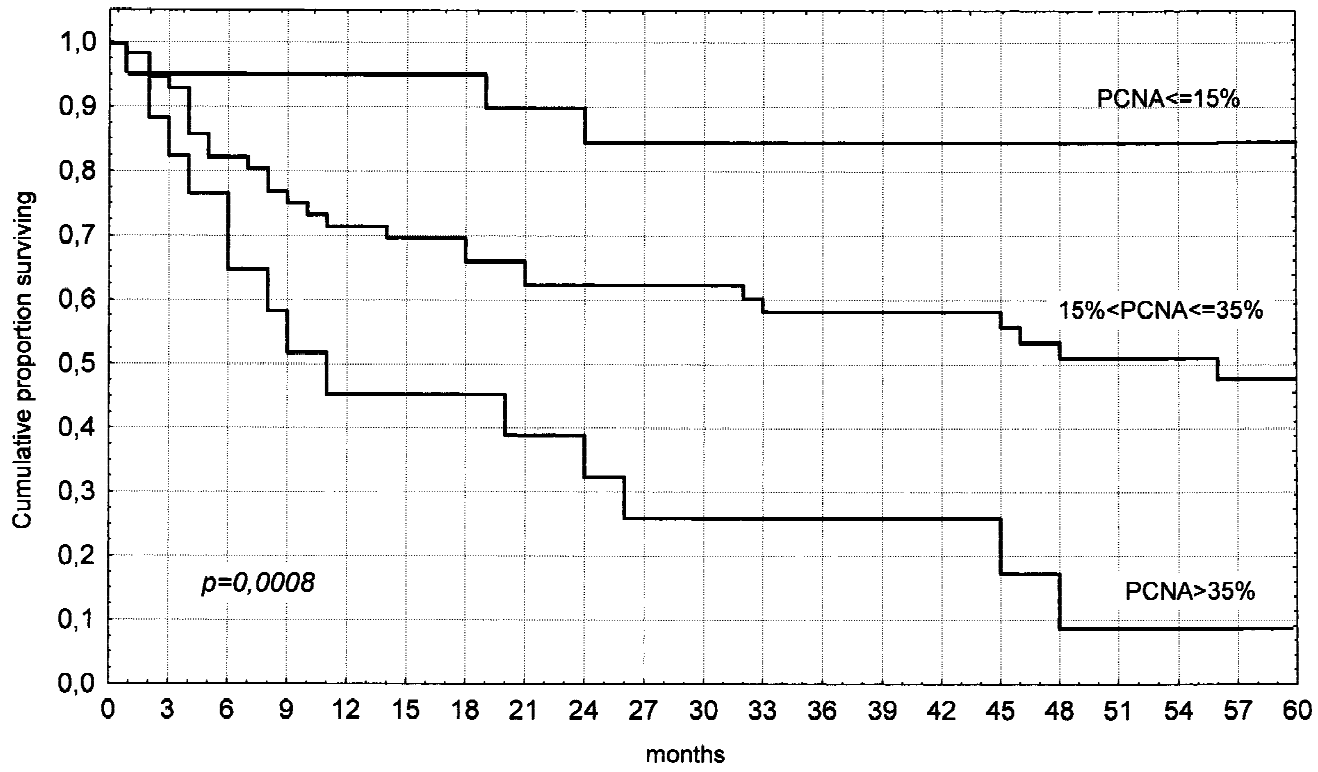

Fig. 4. Disease-free survival curves for 20 patients with PCNA index ⩽15%, 56 patients with PCNA index >15% and ⩽35%, and 17 patients with PCNA index >35%. *P* values taken from log-rank test comparing three survival curves.

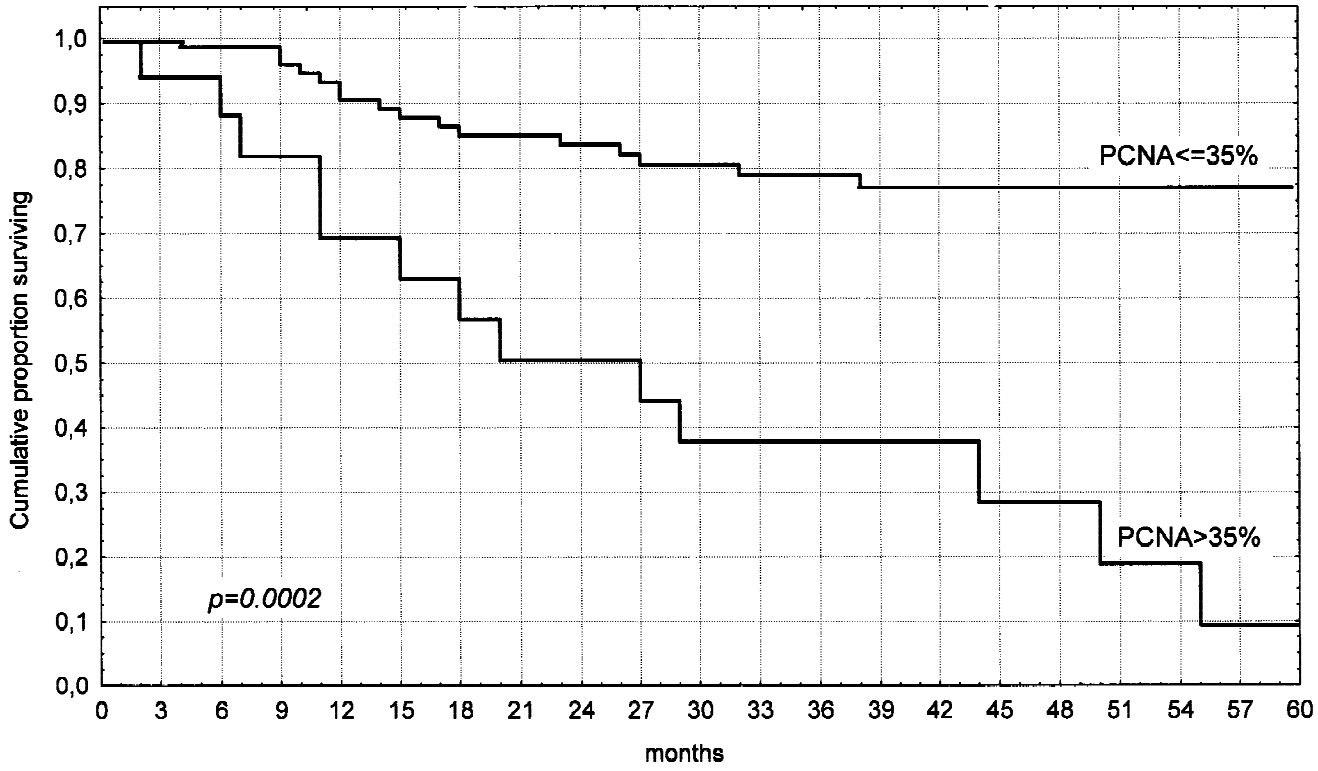

Fig. 5. Overall survival curves for 76 patients with PCNA index ⩽35% and 17 patients with PCNA index >35%. *P* values taken from log-rank test comparing two curves.

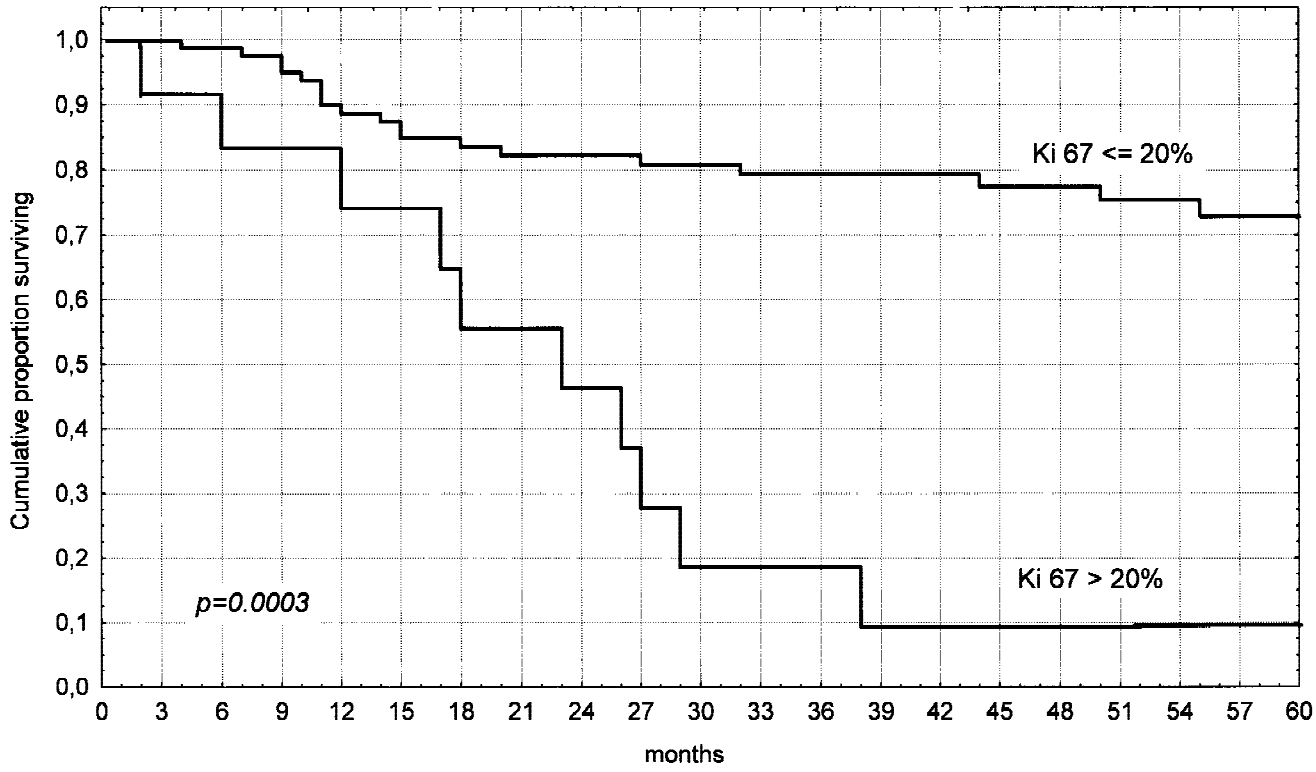

Fig. 6. Overall survival curves for 81 patients with Ki-67 index ⩽20% and 12 patients with Ki-67 index >20%. *P* value taken from log-rank test comparing two survival curves.

cases, therefore their prognostic role and association with other variables studied could not be evaluated. The role of atypical and common acquired nevi, as precursor lesions of malignat melanoma, seems to be well documented but their prognostic value is still an open issue [42–45].

Presence of ulceration and vascular invasion were strongly associated with tumor stage, Breslow thickness, Clark level, and tumor type. They influenced clinical outcome of melanomas studied, but none of them constituted an independent prognostic factor. Other studies demonstrated that vascular invasion associated with both lymphatic and hematogenous spread was interrelated with tumor thickness and diameter and provided prognostic information in relation to survival of melanoma patients [13].

Clemente et al. [9] demonstrated prognostic value of lymphocytic infiltration of malignant melanoma. In the present study, lymphoplasmocytic infiltrate was unrelated to clinical outcome, although its correlation with Clark level and vascular invasion could be demonstrated. Minimal surgical margin of resected lesion was interrelated with patients survival; this result is contrary to other data [46].

S100 protein, HMB 45, and vimentin demonstrated by immunohistochemistry in melanoma cells are of diagnostic usefulness. Their role as predictors of clinical outcome remains obscure; however, some results point to their prognostic value [47,48]. In the present study, only HMB 45 constituted an independent predictor of disease-free survival, although it was not associated with any parameter studied. The two other markers had no influence on clinical outcome.

Proliferative Parameters

In malignant melanomas, expression of p53 protein may be a late event in tumor progression [26]. Expression of p53 protein in melanoma cells was associated with a number of unfavorable prognostic indicators [14,17,22–26] and its value as a predictor of poor clinical outcome was demonstrated [22,25]. In our study, expression of p53 protein was neither associated with other variables nor influenced the outcome of malignant melanoma. However, for evaluation of p53 protein, antibody only for one epitope of this antigen was used and it is possible that a broader panel of antibodies could provide a different result.

Indicators of proliferative activity provided valuable clinical information in malignant melanoma. Expression of Ki-67 (MIB 1) helped to identify melanomas of high metastatic potential [20,21] and was associated with poor survival [18,21]. Expression of PCNA was useful in prediction of locoregional and distant metastases [14,15]. In the present study, mitotic index and PCNA index were

correlated with tumor stage and all indicators of proliferative activity were strongly interrelated with invasion when considered in categories of Breslow and/or Clark. Expression of both PCNA and Ki-67/MIB 1 strongly influenced disease-free and/or overall survival as independent prognostic factors.

In conclusion, our study demonstrated independent prognostic significance of stage, tumor thickness, the expression of proliferative antigens, and HMB 45 in melanoma patients. These variables, except for HMB 45 expression, were associated with other parameters, therefore in detailed characterization of malignant melanoma many prognostic parameters should be considered.

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